J. Res. Trad. Medicine | Nov-Dec 2015 | Volume 1 | Issue 1

Original Article - Pharmacological Research

Effect of *Swarna Bhasma* on Memory and Learning in Swiss Albino Mice

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Received: 8/11/2015 Revised: 15/11/2015 Accepted: 17/11/2015

Abstract

Background: Administration of gold in children is a popular practice in Ayurveda. It is stated that pure gold if administered along with honey and ghee for a period of 6 months will enable the infant to remember things which are just heard. **Aim:** The present study was conducted to evaluate the effect of *Swarna Bhasma* on memory and learning against hyoscine/ scopolamine induced amnesia in albino mice. **Materials & Methods**: Young Swiss albino mice of both sex divided into three groups were used in the study. The trial drug contained *Swarna Bhasma*, honey and ghee whereas adjuvant drug contained only ghee and honey which were made into drops from and directly administered in 2 groups of mice and the other group was kept as the normal control. Elevated plus maze was used to assess the effect of test drugs on transfer latency which is interpreted as effect on learning and memory. Student's t' test was used to analyse the data and the minimal level of significance was identified at $p < 0.05$. **Results**: The trial drug showed significant decrease in transfer latency ($p<0.05$) both on 2nd and 3rd day of the study when compared with initial values and the control group. **Conclusion**: *Swarna Bhasma* exhibited significant therapeutic effects on memory and learning in albino mice.

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Keywords: *Elevated Plus Maze, Gold, Learning, Memory, Swarna Bhasma, Swarnaprashana.*

Introduction

Administration of gold in therapeutics is an age old practice, especially in Ayurveda. In classics the term *Swarnaprashana* is used to indicate administration of gold internally in a semi solid form. It is stated that if gold is administered for a period of 1 month the infant will become highly intelligent and if administered for 6 months the infant will be able to recall things which are just heard by him/ her. It has been mentioned that gold should be administered along with ghee and honey [1]. There is clear cut explanation that gold should be purified before internal administration as impure gold can adversely affect health of an individual and cause serious complications[2]. *Swarna Bhasma* (ash of gold) is the incinerated form gold metal that is indicated for internal administration [3]. Effects of *Swarna Bhasma* on memory, grasping power and diseases caused due to fear, sorrow, anger and mental stress have been quoted in texts of Ayurveda [4]. Thus in present study trial drug (mixture of *Swarna Bhasma*, honey and ghee) and adjuvant drug (the mixture of honey and ghee) were tested for their therapeutic efficacy in established experimental models representing different aspects of memory and learning to provide scientific basis for their further therapeutic applications.

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Materials and Methods

Animals: Young healthy Swiss albino mice (both male and female) weighing about 26 ± 4 g were used for the experimentation. The animals were obtained from Animal house attached to IPGT & RA, Gujarat Ayurved University, Jamnagar. The experimental protocols were approved by Institutional Animal Ethics Committee (IAEC/10/2012/02) in accordance with the guidelines formulated by CPCSEA, India.

Husbandry conditions and diet of all the animals were maintained according to standard protocols. All the selected animals were kept for acclimatization for 15 days before experimentation. The animals were fasted overnight before the experimentation. The animals were marked with saturated picric acid for proper identification.

Preparation of drugs:
The trial drug and the adjuvant drug were prepared in the laboratory of Dept. of Kaumarabhritya, IPGT & RA, Gujarat Ayurved University, Jamnagar. Sophisticated analysis report of the *Swarna Bhasma* which was performed before the preparation of trial drug revealed presence of 52.33% elemental gold in the sample. The XRD pattern of *Swarna Bhasma* sample reflected gold metal as the major phase [5].

The adult dose of *Swarna Bhasma* was taken as 30 mg [6] and the same was considered for the study. It was triturated along with ghee and honey in a specific proportion to prepare the trial drug into drops form. The adjuvant drug contained only honey and ghee in the same proportion as that of trial drug.

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As both of the formulations were in drops form, there was no necessity to prepare stock solution for administration in animals, but directly fed in the fixed dose.

Dose fixation: Dose of the drug in animals was calculated by extrapolating the human therapeutic dose to mice on the basis of body surface area ratio (conversion factor 0.0026) using the table of Paget and Barnes (1964) [7].

a)Trial drug:
Mice dose = Therapeutic human dose × conversion factor for mouse of 20 g

= 30mg (of *Swarna Bhasma*) × 0.0026 = 0.078mg for 20g mouse
= 3.9mg/kg body weight of mice

1 drop of the prepared trial drug contained 0.2mg of *Swarna Bhasma*. Accordingly the dosage was calculated for mice. i.e. 0.0117ml/20g mice = 0.585 ml/kg mice (1 drop = 0.03ml calculated by measuring a drop in the dropper bottle used in the study).

b)Adjuvant drug:
Mice dose of the adjuvant drug was calculated in drops as given above to maintain uniformity in dosage. i.e. 0.0117ml/20g mice = 0.585 ml/kg mice .

Pharmacological drug administration:
Buscopan® Tablets (Hyoscine Butylbromide – 10mg) manufactured by Zydus Healthcare, Batch No. ZHK3564 was used in the present study. It was administered in mice as intra peritoneal injection following the standardised methods [8],[9],[10].

Experimental Protocol:
To assess the effect of trial drug on Memory and Learning [11] the following protocol was used. Young Swiss albino mice of body weight ranging from 26 ± 4 g were used as experimental animals. The selected animals were divided in to three different groups. First group was kept as control for comparison while to second and third groups trial and the adjuvant drugs were administered respectively in the dos of 0.585 ml/kg.

The total experimental procedure was carried out for three consecutive days. Daily the drugs were administered to the animals orally according to the grouping one hour prior to exposure to elevated plus maze. The test was carried out using an elevated plus maze for mice. The plus-maze apparatus consists of two open arms (16 × 5 cm) and two closed arms (16 × 5 × 12 cm) having an open roof and is elevated to 25 cm from the floor. The transfer latency, which is the elapsed time between the time at which the animal is placed in the open arm and the time at which all its legs have crossed the line into the enclosed arm was noted in this test.

On the first day of the trial, the animals were placed individually at the end of either of the open arm. If the animals do not enter an enclosed arm within 180 seconds, it was pushed on the back into one enclosed arm and the transfer latency was given as 180 seconds. The animal was allowed to move freely to explore the apparatus for some time. The transfer latency was again recorded 24 hour after first exposure (on 2nd day).

The transfer latency on the first day trial serve as acquisition (learning) and the retention/consolidation (memory) was examined on 2nd day, 24 hour after first exposure. Further on third day, one hour after administration of drugs, Hyoscine butyl bromide tablet (5 mg/kg, ip) was administered in all groups to induce amnesia. After 1 hour the animals were exposed to elevated plus maze and the transfer latency was recorded. The difference between this transfer latency and that of previous day served as the index of cognition.

Statistical analysis: The data generated during the study were subjected to student's t' test for paired and unpaired data to assess the statistical significance between the groups. 'p' value less than 0.05 was considered as statistically significant.

Results

The data in the table 1 represents the effect of test formulations on Hyoscine induced amnesia in mice. In control group there was decrease in the transfer latency on second day in comparison to initial value which was not significant. There was significant decrease in transfer latency on the second day in trial and adjuvant drugs treated mice in comparison to the initial values.

Trial drug showed marked decrease ($p<0.05$) in transfer latency on second day in comparison to control group. Only trial drug showed statistically significant ($p<0.05$) reduction in transfer latency on third day after Hyoscine administration in comparison to control group. The adjuvant drug did not show any significant changes on transfer latency on comparison with control drug on both second and third day. However on comparison between trial and adjuvant drugs there was no statistically significant difference.

Discussion

Acharya Kashyapa, the father of Ayurvedic paediatrics has explained *Swarnaprashana* under the context of *Lehana* (Electuaries) which depicts the administration of various herbal drugs, ghee preparations and gold alone as supplementary feeds in infants wherein precise indications are stated [12]. Administration of gold along with other herbs is also explained in the context of *Jatakarma Samskara* [13](basic new born care in Ayurveda). In brief the word *Swarnaprashana* means the consumption of gold by means of licking. This unique formula has been explained specifically by *Acharya* Kashyapa wherein gold should be triturated along with water, honey and ghee on a pre-washed and clean stone facing eastern direction and made the *Shishu* (infant) lick the same. It is said that if gold is administered in such a way for 1 month, the baby will become *Parama Medhavi* (highly intelligent)[1]. The word *Medha* indicates a type of *Buddhi* [14] (intelligence). It is also defined as the ability to grasp the knowledge and retain the same [15]. An individual is able to retain the things which he/she has learned or experienced. The assessment of retention power of an individual can be done through his/her grasping capacity (*Medham Grahanena*) [16]. Recollection of facts which was seen, heard or experienced is known as *Smruti* [17] (memory).The present study was an attempt to study the effect of *Swarna Bhasma* on memory and learning in order to assess *Medha* at a pre-clinical level. This protocol helped to assess the effect of the formulation on *Medha* as told by *Acharya* Kashyapa. Intellectual ability is a complex phenomenon, influenced by factors both environmental and biological. Used in its broadest sense, intelligence is what people use to learn, remember, solve problems and in general deal effectively with the world around them [18]. Memory is an organism's mental ability to store, retain and recall information. It provides the essential substrate for the cognitive activities that define human experience and allows one to connect the present moment to what came before. The process of retaining and reproducing what has been learnt is known as rememberance[19]. Thus memory can be considered as the factor contributing to intelligence. The present study attempted to demonstrate the four main elements involved in memory namely learning, retention, recall and recognition.

The effects of drugs on behaviour such as on the phenomena of learning, memory, wakefulness, sleep, and drug addiction are analysed in behavioural pharmacology. It also deals with the behavioural consequences of experimental intervention in enzyme activity, brain neurotransmitter levels and metabolism [20]. Elevated plus-maze (EPM) used in the present study can be considered as an exteroceptive behavioural model for evaluation of memory in mice. It demonstrated the effect of test drugs on transfer latency. EPM is an established model for testing memory and learning in mice based on changes in transfer latency [21].

Memory enhancing drugs reverse the effect of the amnesic drug. Scopolamine which is also called Hyoscine, is a hallucinogenic alkaloid drug obtained from plants of the family Solanaceae. It is a very potent psychoactive drug, which is used as a standard drug for inducing amnesia in mammals.

Table 1: Effect of Trial and Adjuvant drugs on Hyoscine induced amnesia during elevated plus maze test in mice

Groups	**Transfer Latency to closed arm (sec)**						
	Initial on 1st day	**After 24 hr on 2nd day**	**% change to initial**	**% change to control**	**1 hr after Hyoscine on 3rd day**	**% change to initial**	**% change to control**
Control	70.00±12.82	61.00±14.45	12.86↓	--	44.00±14.50	37.14↓	--
Trial Drug	60.33±14.66	**23.33±5.71**$^{\#*}$	61.33↓	61.75↓	**13.00±1.89**$^{\#*}$	78.45↓	62.88↓
Adjuvant drug	87.50±2.50	**44.83±11.97****	48.70↓	26.50↓	**25.16±13.11****	71.25↓	42.81↓

Data: Mean ± SEM, ↓ - Decrease, $^{\#}p<0.05$ when compared with control group
$^{*}p<0.05$, $^{**}p<0.01$ when compared with initial values

The effects are generally interpreted as a cholinergic deficit and related to the fact that acetylcholine is involved in memory functions. The use of scopolamine as a pharmacological model of cholinergic amnesia is a very popular method as quoted in various studies [22]. Since scopolamine-induced amnesia was proposed to be due to blockade of cholinergic neurotransmission, this substance is used to model the cognitive deficits that could be observed in dementia and aging [23]. The alkaloid L-(-)-scopolamine [L-(-)-hyoscine] competitively inhibits muscarinic receptors for acetylcholine and acts as a non-selective muscarinic antagonist, producing both peripheral anti-muscarinic properties and central sedative, antiemetic, and amnesic effects[24].

In the present study Hyoscine Butylbromide was used to model aspects of amnesia in mice. The effect of trial and adjuvant drugs on memory was evaluated by noting their performance on elevated plus maze. The experimental protocol of three consecutive days was followed in which on first day acquisition was noted and on the second day, the retention/consolidation (memory). The significant decrease in transfer latency observed in trial drug treated group on the second day clearly shows the establishment of memory about exposure of the animals to the elevated plus maze instrument which was not significant in control group. This clarifies that the trial drug could improve memory significantly. On third day, effect of drugs on Hyoscine induced amnesia was noted. The latency of first entry into closed arm in control group was found to be increased due to amnesic property of Hyoscine. The significant decrease in transfer latency showed by trial group when compared to control group signifies the reversal of amnesic effect.

In a study calcined preparations of gold (*Swarna Bhasma)* used in Ayurveda, along with other oral gold preparation used in modern medicine were subjected to a set of more than 30 screening tests for general neuropsychopharmacological effects, cognitive functions, anti-depressant, anxiolytic, neuroleptic and serenic activities as also for the effects on endurance and fatigue. Nootropic effects in rats and mice against both active and passive avoidance models, anxiolytic effects observed by Vogel's conflict test and elevated plus maze studies (in gold preparations) [25] were observed. Another experimental study has reported anxiolytic activity of *Swarna Bhasma* in mice [26]. A Pharmacological study conducted on young and aged mice has suggested nootropic and anti-AChE activity of *Swaranaprashana* where *Swarna Bhasma* was administered along with ghee and honey similar to the present study but in different dosage [27]. Even no comparison with the adjuvant group was done in that study.

The results in the present study also go hand in hand with the previous reports. Significant reduction of transfer latency brought about by the trial drug might be due to inhibition of breakdown of Acetylcholine

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and thus increasing the level and duration of action of the same. Acetylcholine is considered as the most important neurotransmitter involved in the regulation of cognitive functions. AChE inhibits the acetyl cholinesterase enzyme from breaking down acetylcholine, thereby increasing both the level and duration of action of the neurotransmitter acetylcholine in the central cholinergic pathways [28].

Conclusion

The present study demonstrated the anti-amnesic activity of the *Swarna Bhasma* by producing antagonism to hyoscine induced amnesia and increasing the performance of learning and memory in mice. Although animal models have limitations in replication of normal and abnormal human mind, the results of the study are encouraging.

Acknowledgement

The authors would like to acknowledge the Vice Chancellor of Gujarat Ayurved University, Prof. P.K. Prajapati, Director of IPGT & RA, Jamnagar and Prof. M. S. Baghel, Former Director of IPGT & RA, Jamnagar for their kind support in the study.

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How to Cite the Article: KB Jyothy et al, Effect of *Swarna Bhasma* on Memory and Learning in Swiss Albino Mice. **J. Res. Trad. Medicine 2015;1(1): 23-28**

Source of Support: IPGT & RA, GAU, Jamnagar

Conflict of Interest: Nil